Carotenoid transfer to oil upon high pressure homogenisation of tomato and carrot based matrices

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- HPH is efficient in transferring carotenoids to oil
- Carotenoid transfer to oil upon HPH is negatively correlated with particle size
- Selective transfer of a particular carotenoid depends on its hydrophobicity
- Cell walls in tomato cell clusters limit carotenoid transfer to oil upon HPH

Abstract

The transfer of carotenoids into the oil phase during digestion is limited by matrix related factors that hamper carotenoid release and the conditions of low gastric acidity that limit carotenoid solubility. Therefore, the aim of this study was to investigate the use of high pressure homogenisation (HPH) as a driving force to favor carotenoid transfer to oil before digestion. The level of bioencapsulation and carotenoid hydrophobicity were investigated as factors that may influence carotenoid transfer from tomato and carrot based matrices to oil upon HPH. The results indicated that the selective transfer of a particular carotenoid depended on its hydrophobicity. The cell wall in tomato cell clusters represents a limiting factor for carotenoid transfer. Overall, the findings indicate that HPH is efficient in transferring carotenoids to the oil phase and this can be crucial to improve the nutritional quality of carrot and tomato-based products.

Keywords: High pressure homogenisation, carotenoid transfer, lycopene, β-carotene, α-carotene, structural barriers.

1. Introduction

There is evidence that dietary carotenoids from fruits and vegetables are beneficial to human health (Margalit et al., 2012; Mayne, 2013; Zaini et al., 2012). Nevertheless, it is known that despite their prevalence in fruits and vegetables, the absorption of carotenoids during digestion can be low and highly variable depending on the diet and host-related factors (Faulks & Southon, 2005). This is because in order to confer their health effects, carotenoids must first be released from the matrix, solubilised in the lipid phase of chyme followed by transfer into mixed micelles in the small intestine before being taken up by the body and finally reach their site of action (Castenmiller et al., 1999). As a result, the health benefits of carotenoids are strongly dependent on their bioaccessibility (i.e. fraction of an ingested nutrient that is released from the food matrix and made available for intestinal absorption). It is apparent that the different steps involved in the carotenoid absorption process are conditioned by different factors that can be controlled/modified in order to improve carotenoid bioaccessibility (Fernández-García et al., 2012). In this context several studies in the past decades have focused on understanding the factors determining the bioaccessibility of carotenoids from fruit and vegetable materials.

It is well established that processing, thermal and/or mechanical can be exploited to facilitate carotenoid release from the matrix and as a consequence, influence carotenoid bioaccessibility (Colle, et al., 2010a; Knockaert et al., 2012a; Panozzo et al., 2013; Svelander et al., 2011). Nevertheless, processing may lead also to carotenoid *trans-cis* isomerisation. The *cis* isomers possess different biological properties such as decreased pro-vitamin A activity and altered antioxidant capacity as compared to the respective *trans* isomers (Schieber & Carle, 2005). It is important to note that while processing disrupts cellular structures and organelle membranes which can facilitate carotenoid release (Van Buggenhout et al., 2010), some modifications of the matrix have been reported to actually entrap

63 carotenoids limiting their release during digestion. In addition, carotenoid release depends on
64 the structural barriers (i.e. chromoplast sub-structures and cell walls) naturally present in the
65 matrix (Palmero et al., 2013). Moreover, some extrinsic factors, such as the co-ingestion of
66 lipids, have been demonstrated to largely affect carotenoid bioaccessibility (Colle et al.,
67 2013; Huo et al., 2007; Salvia-Trujillo et al., 2013). In this context, carotenoid solubilisation
68 into the oil phase has been highlighted as one of the critical steps in their absorption (Hedrén,
69 Diaz, & Svanberg, 2002). Thus, the type of carotenoid and its solubility in the lipid phase
70 play an important role in determining carotenoid bioaccessibility. For instance, a lower
71 bioaccessibility of lycopene compared to β-carotene was reported and attributed to be related
72 to the lower solubility of the former in dietary lipid compared to the latter (Svelander et al.,
73 2011). Recently, a study by Palmero, Panozzo, Simatupang, Hendrickx, & Van Loey (2014)
74 demonstrated the strong dependency of carotenoid transfer efficiency during digestion from
75 the matrix into the oil phase on the level of bio-encapsulation as well as carotenoid
76 hydrophobicity. This implies that the positive effects that can be realized by employing
77 processing to enhance carotenoid release are diminished greatly by interplay of the factors
78 that are controlling the movement of carotenoids from the matrix to the oil during digestion.
79 These challenges are arising mainly from their low water solubility (due to their highly
80 lipophilic nature), high melting point (due their existence in crystalline form) and poor
81 chemical stability (due to the conjugated system of double bonds) (Boon et al., 2010).
82 From the above, processing fruit and vegetable based matrices in the presence of oil can be
83 exploited to facilitate transfer of carotenoids to an oil phase as a means of introducing the
84 lipid phase prior to ingestion. In this respect, high pressure homogenisation (HPH) can be a
85 useful tool to facilitate the transfer of carotenoids from their natural location in the matrix to
86 the oil phase. This transfer may for instance be affected by diffusion or by turbulent mixing
87 or generally by the combined action of diffusion, turbulence and convection. In general, the

mechanism of mass transfer depends on the dynamics of the system in which it occurs (Bravo, 2011). By recognizing that mass can be transferred by random molecular motion in quiescent fluids, aided by the dynamic characteristics of the flow (Bravo, 2011), the applied homogenisation pressure during HPH can be exploited as the driving force for carotenoid transfer into oil. This can be useful particularly for those fruit and vegetable based matrices such as tomato where the formation of a fibre network that entraps lycopene in the matrix following processing, are carried over through the digestion process. In this way, matrix related factors that hamper carotenoid release (Castenmiller et al., 1999) and the conditions of low gastric acidity that limit carotenoid solubility into the oil phase during digestion (Rich, Fillery-Travis, & Parker, 1998) can be circumvented. Nonetheless, the potential of HPH to facilitate carotenoid transfer to oil has not been reported. This could potentially pave the way to a new and/or different applications in the context of process design for targeted bioactive compounds such as carotenoids in fruit and vegetable processing. In this regard, it is crucial to establish whether factors such as carotenoid hydrophobicity and level of encapsulation which have been highlighted above as influencing the transfer efficiency into oil during digestion are persisting during processing. Therefore, the present work evaluated the transfer of lycopene, *β*-carotene and *α*-carotene from tomato and carrot based matrices to oil as influenced by HPH.

2. Materials and Methods

2.1 Materials

All chemicals and reagents used were of analytical or HPLC-grade. L-*α*-phosphatidylcholine and carotenoid standards (all-*trans* lycopene, all-*trans β*-carotene and all-*trans α*-carotene) were purchased from Sigma-Aldrich (Borne, Belgium). Olive oil (extra virgin) was kindly donated by Vandemoortele (Ghent, Belgium). Red ripe tomatoes (*Lycopersicon esculentum*

cv Prunus) and orange carrots (*Daucus carota cv* Nerac) were obtained fresh from a local shop in Belgium and stored at 4 °C for 1 day prior to use.

2.2 Experimental set up

The effect of high pressure homogenisation on carotenoid transfer from tomato and carrot matrices to oil was investigated. At first carrot and tomato matrices were decomposed into chromoplast-enriched and cell clusters fractions, as described further in the text. In this way, different physical barriers that hinder carotenoid release (chromoplast substructure and cell walls) were considered. The different fractions isolated from each matrix were mixed with an oil-in-water emulsion (5% oil) and high pressure homogenised at different pressure levels (10, 30, 50, 70, 100 MPa). Non-homogenised samples were considered as control samples. Treated and untreated samples were then ultra-centrifuged (Beckman Optima XPN-100 Ultracentrifuge, Brea, CA, USA) at 65 000 g for 1 hour at 4 °C in order to recover the oil. The recovered oil was analysed for carotenoid content. In particular, two types of carotenoids were quantified in the isolated fractions from each matrix, namely all-*trans* lycopene and all-*trans* β-carotene in tomato, and all-*trans* β-carotene and all-*trans* α-carotene in carrot based system. Carotenoid content in the recovered oil from each fraction at each pressure level was compared with carotenoid content in the untreated isolated fractions. The amount of carotenoids transferred to the oil phase was expressed as either absolute concentration, i.e. µg carotenoid/ g oil, or relative amount, i.e. % carotenoid transfer according to equation 1:

$$\text{Transfer (\%)} = \frac{\text{µg carotenoid in recovered oil}}{\text{µg carotenoid in the control}} \times 100 \qquad (1)$$

Furthermore, particle size distributions of the samples were investigated in order to gain insight into the relation between particle size reduction during high pressure homogenisation and carotenoid transfer to oil.

2.3 Sample preparation

2.3.1 Oil-in-water emulsion

137 Oil-in-water emulsions were prepared by mixing 5% olive oil to 1% L-*α*-phosphatidylcholine
138 in deionized water at 9500 rpm for 10 min (UltraTurrax, IKA-Werke GMBH and CO.KG,
139 Staufen, Germany). The mixture was then immediately homogenised at 100 MPa for one
140 cycle (Panda 2 K, Gea Niro Soavi, Parma, Italy).
141 2.3.2 Chromoplast fraction
142 The chromoplast-enriched fraction from tomatoes or carrots was obtained according to the
143 methods described by Palmero et al. (2013). The vegetables were first washed in deionized
144 water. Tomatoes or peeled carrots were cut into pieces and mixed (Waring Commercial,
145 Torrington, CT, USA) at low speed for 5 s with 50% 0.05 M ethylenediaminetetraacetic acid
146 (EDTA) solution. The obtained purees were gently filtrated using cheesecloth and further
147 centrifuged (Beckman, J2-HS Centrifuge, Palo Alto, CA, USA) at 27200 *g* for 30 min at 4
148 °C. The pellet was re-suspended in 100 ml deionized water and represented the chromoplasts
149 enriched fraction.
150 2.3.3 Cell cluster fractions
151 Cell cluster fractions were prepared according to the procedures described by Palmero et al.
152 (2013). Firstly, tomato or carrot purees were obtained. In the case of tomatoes, the pieces
153 were mixed (Büchi B-400 mixer, Flawil, Switzerland) three times for 5 s and sieved (1 mm)
154 to remove seeds. Carrot puree was obtained by mixing (Waring Commercial, Torrington, CT,
155 USA) the carrot pieces with 50% deionized water for 1 min. The obtained purees were then
156 sieved using wet sieving equipment (Retsch AS200, Haan, Germany). The fractions between
157 40–250 and 160–500 μm were collected representing the carrot and tomato cell cluster
158 fractions, respectively (Lemmens et al., 2009; Palmero et al., 2013).
159 2.4 High pressure homogenisation
160 The homogeniser (Panda 2K, Gea Niro Soavi, Parma, Italy) used in this experiment was
161 connected at the outlet to a heat exchanger at a pre-set temperature of 4 °C in order to

withdraw the heat generated during the passage of the sample through the homogenisation valve. Also the sample inlet was thermostated at 4 °C. Cell clusters and chromoplast fractions from carrots or tomatoes were separately mixed with the oil phase in a ratio (fraction-emulsion) of 1:10 (w/v). It is important to note that in the present work, the oil phase was in the form of a 5% oil-in-water emulsion in order to have comparable oil droplet size distributions in the samples since carotenoid transfer from matrix to oil can depend on the size of lipid droplets (Tyssandier et al., 2003). The samples were immediately homogenised at 10, 30, 50, 70 and 100 MPa for one cycle. After the homogenisation step, samples were kept at 4 °C until phase separation by centrifugation at (Beckman Optima XPN-100 Ultracentrifuge , Brea, CA, USA) at 65 000 *g* for 1 hour at 4 °C in order to recover the oil. For each pressure level, the treatment was repeated twice and carotenoid analyses on the recovered oil done in triplicate. Next to this, a control sample was prepared to which no homogenisation was applied.

2.5 Particle size measurements

The particle size distribution was measured using laser diffraction particle size analyser (Beckman Coulter LS 13 320, Miami, FL, USA). The instrument measures particle sizes in the range of 0.04 to 2000 µm. A small amount of sample was poured into a stirred tank, filled with deionized water, and pumped into the measurement cell. The intensity profile of the scattered light was used to calculate the volumetric particle size distributions (PSD)s using the Fraunhofer optical model by use of the instrument software (Jamsazzadeh Kermani et al., 2015). The volume-based ($d_{(4,3)}$) and area-based ($d_{(3,2)}$) diameters were obtained for each sample, according to equations 2 and 3:

$$d_{(4,3)} = \sum_i^n n_i d_i^4 / \sum_i^n n_i d_i^3 \qquad (2)$$

$$d_{(3,2)} = \sum_i^n n_i d_i^3 / \sum_i^n n_i d_i^2 \qquad (3)$$

where n_i is the number of particles of diameters d_i (in micrometres). Since $d_{(4,3)}$ is greatly influenced by large particles, whereas $d_{(3,2)}$ can be sensitive to the fraction of smaller particles present, both equivalent diameters were evaluated (Bengtsson, Wikberg, & Tornberg, 2011; Cortés-Muñoz, Chevalier-Lucia, & Dumay, 2009). The parameter $d_{v,\,0.5}$ which indicates the particle diameter at which 50 % of the volume of the particles have a smaller diameter was also evaluated (Moelants et al., 2011). All analyses were carried out in duplicate.

2.6 Carotenoid concentration

The untreated isolated fractions and the recovered oil from the treated samples were immediately subjected to the carotenoid extraction procedure in order to determine the initial amount of carotenoids, and the amount of carotenoids transferred to the oil phase during HPH, respectively. Carotenoids were extracted following the procedure described by Sadler et al. (1990). The procedure was performed by mixing 1 ml (chromoplast fraction) or 1 g (cell cluster fraction) with 25 ml of the extraction solution [hexane/acetone/ethanol (50:25:25 v/v/v) containing 0.1% of butylated hydroxytoluene (BHT)] and 1 g of NaCl. Afterwards, the mixture was stirred for 20 min at 4 °C, followed by the addition of 7.5 ml of reagent grade water (18.2 MΩ·cm). For the recovered oil, 0.5 g oil was mixed with 10 ml of the extraction solution and 0.1 g NaCl, stirred for 20 min at 4 °C, followed by addition of 3 ml reagent grade water. The mixtures were mixed for 10 more minutes at 4 °C. The mixtures were then placed in separation funnels (or glass tubes in case of recovered oil) to collect the organic phase. The isolated organic phase was filtered (Chromafil PET filters, 0.2 µm pore size-25 mm diameter) and transferred into a dark vial for HPLC analysis.

The identification and quantification of carotenoids were performed using a HPLC system equipped with a C_{30}-column (3µm×150mm×4.6mm, YMC Europe, Dinslaken, Belgium) and a diode array detector (Agilent Technologies 1200 Series, Dinslaken, Belgium). The temperature of the column was kept constant at 25 °C during the analyses. A linear gradient,

using methanol (A), methyl tert-butyl ether (B) and reagent grade water (18.2MΩ·cm) (C), was applied. The starting conditions were 81% A, 15% B and 4% C and the ending conditions corresponded to 16% A, 80% B and 4% C. The flow rate was set at 1 ml/min and the gradient was built up in 44 min. Identification was performed at 472 nm for all-*trans* lycopene and the *cis* isomers and at 450 nm for all-*trans* *β*-carotene and the *cis* isomers, as well as all-*trans* *α*-carotene on the basis of retention times and spectral characteristics of pure standards as described by Colle et al. (2010a) and Lemmens et al. (2009). All-*trans* lycopene, all-*trans* *β*-carotene and all-*trans* *α*-carotene were quantified with the use of the corresponding calibration curves. Based on the chromatograms obtained, *cis* isomers were not detected in isolated fractions nor in the recovered oil from high pressure homogenised samples as was previously reported (Edwards et al., 2002; Colle et al., 2010b; Knockaert et al., 2012b). In this work, *β*-carotene, *α*-carotene and lycopene refers to the all-*trans* forms.

2.7 Statistical analysis

In order to evaluate significant differences among percentage carotenoid transfer, carotenoid concentrations in the recovered oil, and particle size reduction in each fraction after high pressure homogenisation, a Tukey's Studentised Range Test was applied by use of software package SAS version 9.4 (Carry, NC, USA). The level of significance was set at $P<0.05$. The mean value of two measurements from two independent treatments was calculated for particle size analyses while for carotenoid concentrations and percentage transfer, analyses were carried out in triplicate. A linear regression procedure was also applied, and parameters estimated using the same software package in order to evaluate the relationship between carotenoid transfer to oil and particle size reduction during high pressure homogenisation.

3. Results and discussion

3.1 Effect of HPH on carotenoid transfer to oil

The recovered oil from both treated and untreated fractions of carrots and tomatoes was analysed for carotenoid content. The results are reported as µg carotenoid/g oil as a function of applied homogenisation pressure in Table 1. The oil recovered from the untreated (control) cell clusters and chromoplast fractions of both carrot and tomato had significantly lower carotenoid concentration than the oil recovered from to the high pressure homogenised samples. Nevertheless, within the control samples concentrations of carotenoids was always higher in the oil recovered from the chromoplast compared to the cell cluster fractions. This indicates that when no driving force is applied, the mass transfer of carotenoids to the oil phase is rather limited and depends on the level of carotenoid bio-encapsulation.

With respect to the treated samples, in both matrices, carotenoid concentration in the oil increased initially and evolved to equilibrium with increasing homogenisation intensity. Generally, no significant differences in carotenoid concentrations in the recovered oil were found at 50, 70 and 100 MPa, with the exception of β-carotene in carrot cell cluster fraction where only 70 and 100 MPa were not significantly different. Similarly to what was observed for the untreated samples, carotenoid concentration in the recovered oil from high pressure homogenised samples was always higher in the chromoplast-enriched compared to the cell cluster fractions. This result was expected as demonstrated in previous studies that a higher amount of carotenoids can be released from the matrix following elimination of cell walls by processing (Palmero et al., 2013). In fact, it was explained that the lack of the cell wall, which was proven to act as an important structural barrier, favours carotenoid release for the chromoplast-enriched fraction compared to the cell cluster fraction. An increase in carotenoid concentration in the oil after HPH has been reported in systems comprising previously thermally treated carrot purees and 5 % olive oil (Svelander et al., 2011). Nevertheless, data reporting the use of HPH as a tool to facilitate carotenoid transfer from fruit and vegetable-based systems to oil is scarce. The increase in carotenoid concentration in the oil after HPH

260 can be attributed to increased shearing and contact between the matrix and the oil phase,
261 which can facilitate the partitioning of the carotenoids from the matrix into oil (Floury,
262 Desrumaux, & Lardières, 2000; Schultz, Wagner, Urban, & Ulrich, 2004).
263 In order to evaluate the selectivity of the different carotenoids for transfer to oil during HPH,
264 concentration ratios in the recovered oil from each fraction as influenced by high pressure
265 homogenisation were calculated from Table 1 (data not shown). In particular, the
266 concentration ratio between β- and α-carotene, and β-carotene and lycopene in the recovered
267 oil from carrot and tomato based samples, respectively, were considered. The carotenoid
268 ratios in the cell cluster fractions of both matrices increased with applied homogenisation
269 pressure and tended towards asymptotic behaviour at higher pressures. The ratios in the
270 chromoplast-enriched fractions of carrot at all pressure levels increased compared to the
271 control sample, but stayed constant as a function of applied homogenisation pressure. On the
272 other hand, the ratios in the treated chromoplast-enriched fractions of tomato were not
273 significantly different from the control sample. Furthermore, the carotenoid ratio values
274 obtained for the cell clusters increased towards those obtained for the chromoplast fractions.
275 The ratio [α-carotene: β-carotene] for untreated carrot cell clusters was in the range of ratios
276 previously reported in literature for carrot purees obtained from typical orange carrot varieties
277 (Edwards et al., 2002; Livny et al., 2003). The ratio can be influenced by processing and
278 handling techniques. For example, Edwards et al. (2002) reported α-carotene: β-carotene
279 ratios of 0.40 in cooked carrot purees compared to 0.34 in raw purees. On the other hand, for
280 tomato there is a wide variability in the ratio [β-carotene: lycopene] in literature since in
281 addition to cultivar type, lycopene content varies significantly with the ripening stage
282 (Baranska, Schütze, & Schulz, 2006). Nevertheless, the ratios [β-carotene: lycopene] were in
283 the same range as reported by Kozukue and Friedman, (2003) and Baranska, Schütze, &
284 Schulz, (2006). Changes in carotenoid concentration ratios with increasing homogenisation

intensity suggests a preference for the transfer to oil of α-carotene over β-carotene in carrot cell clusters and β-carotene over lycopene in tomato cell clusters. This again can be due to the differences in carotenoid hydrophobicity. In addition, carotenoid selectivity for transfer to oil was pressure dependent in the cell clusters. However, this dependence was lost when the cell walls were removed from the system, since a fairly constant carotenoid ratio with increasing homogenisation intensity in the chromoplast-enriched fractions was found.

To compare the efficiency of HPH in assisting the transfer to oil of different carotenoid species, from different fractions and matrices, carotenoid percentage transfer was calculated according to equation 1. Carotenoid percentage transfer to the oil phase as a function of the homogenisation pressure levels is illustrated in Fig 1. Similarly to what observed for carotenoid concentration in the recovered oil (Table 1), in both matrices carotenoid percentage transfer increased with increasing homogenisation pressure and evolved to a plateau. This can be explained by the increase in contact between the matrix and the emulsion due to increased shearing under the applied pressure (Schultz et al., 2004). In general, no significant differences in carotenoid transfer at 50, 70 and 100 MPa were observed. This means that in carrot and tomato, regardless the level of encapsulation, the main changes in carotenoid transfer took place between 10 and 50 MPa, and tended to an asymptotic behaviour between 50 and 100 MPa, as reflected also in Table 1. Moreover, as significantly higher amounts (P<0.05) of carotenoids could be transferred from the chromoplast-enriched fractions to oil than from cell clusters (Table 1), under the present conditions the asymptotic value can be assumed to depend on carotenoid initial concentration in the different fractions, and not on the saturation of the oil phase.

The differences in carotenoid transfer at 50, 70 and 100 MPa between cell clusters and chromoplast fractions within each fraction (Fig 1) was noteworthy, especially in the case of tomato samples. To this regard, Palmero et al. (2013) reported that the presence of the cell

310 wall material in tomato cell clusters plays a different role in influencing carotenoid release as
311 compared to the carrot cell clusters. This observation confirms once again, the crucial role of
312 matrix in governing carotenoid transfer to oil.
313 In the carrot matrix (Fig 1A and Fig 1B), the percentage transfer of α-carotene was always
314 higher than that of β-carotene. For example, at 100 MPa this percentage transfer was
315 73.1±2.7% compared to 62.9±0.9% in the cell clusters fraction. As for the tomato matrix (Fig
316 1B and Fig 1C), within each fraction, the percentage transfer of β-carotene was always higher
317 compared to that of lycopene. For example, at 100 MPa this transfer was 35.0±0.7%
318 compared to 23.7±0.2% in the cell clusters fraction. This was expected due to the differences
319 in the chemical structures of the two carotenoids which in turn can influence their
320 hydrophobicity (Britton, 1995). In fact, lycopene having a linear structure is the most
321 hydrophobic carotenoid (Mortensen, Skibsted, Sampson, Rice-Evans, & Everett, 1997) and
322 therefore, β-carotene could be incorporated into the oil phase more efficiently than lycopene.
323 These results are in line with those of Palmero et al.(2014). These authors also suggested that
324 the different sub-location or association with other molecules of carotenoids might influence
325 carotenoid release and hence transfer to oil during high pressure homogenisation. However,
326 in the present experiment it is not known to what extent the applied pressure modifies the
327 association of carotenoids with other molecules in the matrix.
328 Comparing the chromoplast-enriched fractions from carrot (Fig 1B) and tomato (Fig 1D) and
329 considering the same carotenoid type (i.e. β-carotene), a higher percentage carotenoid transfer
330 at 100 MPa was observed in carrot compared to tomato (78.01±0.47 vs 69.33±1.03%).
331 Although in the same order of magnitude, this can be attributed to differences in the
332 ultrastructure of the chromoplasts from carrot and tomato. The ultrastructure of both carrot
333 and tomato chromoplast has been reported in the literature (Jeffery, Holzenburg, & King,
334 2012; Kim, Rensing, Douglas, & Cheng, 2010). While in carrots carotenoids are accumulated

as large crystals inside the lumina of thylakoid-like structures, in tomato carotenoids are predominantly in the form of crystalloids in membrane-shaped structures (Schweiggert, Steingass, Heller, Esquivel, & Carle, 2011). The present results apparently disagree with the findings of Palmero et al. (2014). According to these authors, carotenoid release from the matrix and subsequent transfers into the oil phase was not affected by differences in the structural characteristics of the chromoplasts sub-structures of tomatoes and red carrots. However, it should be noted that in Palmero et al. (2014) the oil phase was added to the sample after processing, and carotenoid transfer to oil was investigated during static in vitro digestion. By contrast, in the present experiment the oil was processed together with the matrix, and HPH was used as a driving force for carotenoid transfer from the matrix to oil. Overall, results suggest that the benefits of employing HPH as a tool to increase the concentration of carotenoids in oil (and then available for transfer to micelles during digestion) can be best realized if two conditions are met. Firstly, no intact cells should be present in the system, and secondly oil should be added during processing. The latter is particularly important for some carotenoids such as lycopene. The latter has been reported to exhibit limited transfer efficiencies from the matrix to dietary lipids under the conditions of the gastro intestinal tract as a result of entrapment within the matrix and the limited solubility(Palmero et al., 2014; Rich, Fillery-Travis, & Parker, 1998). For example, Palmero et al.(2014) reported lycopene and β-carotene transfer efficiencies from the chromoplast-enriched fractions to oil during in vitro digestion of tomato and red carrot based-fractions of' up to 66 and 56 %, respectively. On the other hand, in the present study, HPH resulted in up to 75 % and more than 80 %, lycopene and β-carotene transfer from the chromoplast-enriched fractions to oil, respectively, indicating that HPH was more efficient in transfering carotenoids to oil than digestion.

3.2 Effect of high pressure homogenisation on particle size distribution

The effect of high pressure homogenisation on the PSD of the untreated and treated cell clusters and chromoplast-enriched fractions from both carrot and tomato is depicted in Fig 2. The PSDs of the untreated cell clusters (Fig 2A and Fig 2C) from both carrot and tomato exhibited a bimodal distribution. The major modes occurred around 250 and 500 µm in carrot and tomato cell clusters, respectively, while the minor modes appeared between 0.5 and 1 µm in both matrices. Based on the particle size, the major and minor modes in both carrot and tomato samples accounted for the cell clusters and the oil droplets size, respectively (Knockaert et al., 2012b; Lemmens, Van Buggenhout, Van Loey, & Hendrickx, 2010). Upon HPH, a shift towards smaller particle dimension of both major and minor modes was observed in the cell clusters of carrot and tomato. In particular, an increase in the percentage volume fraction of the peak corresponding to small particles and a decrease in the percentage volume of the peak corresponding to large particles could be observed upon HPH. The increase in the percentage volume fraction of the oil droplets is a result of the increasing shear stress due to increasing homogenisation pressure which in turn can lead to oil droplet deformation (Floury, Desrumaux, & Lardières, 2000). In addition, the narrowing of the PSD modes indicated an increased uniformity of the sample as a consequence of HPH. Carrot cell clusters were more resistant to disruption upon high pressure homogenisation than tomato cell clusters. Similar results have been reported previously for carrot based matrices (Knockaert et al., 2012a; Lopez-Sanchez et al., 2011; Pickardt, Dongowski, & Kunzek, 2004) and tomato-based matrices (Augusto et al., 2012; Bengtsson, Wikberg, & Tornberg, 2011; Colle et al., 2013).

Regarding the untreated chromoplast-enriched fractions (Fig 2B and Fig 2D), the particle size of both carrot and tomato samples was characterised by a unimodal distribution around 7 and 10 µm. Also in the case on the chromoplast-enriched fraction, a shift towards smaller particle size was observed in both carrot and tomato samples. In this regard, homogenisation at 10

385 MPa was enough to cause particle size disruption and reduce the average particle diameters
386 from 10-15 μm to 1-2 μm (Fig 2B and Fig 2D). Increasing the homogenisation intensity from
387 30 to 100 MPa resulted only in an increase in uniformity of the sample, as indicated by the
388 narrowing of the PSDs (Knockaert et al., 2012b; Kubo et al., 2013). In addition, upon HPH at
389 10 MPa the PSD mode showed the occurrence of a small peak (shoulder) accounting for
390 particles having slightly larger size than the main ones. This peak, which possibly originated
391 from undisrupted particles, decreased with increasing homogenisation intensity.
392 To better estimate the effect of HPH on the particle size of carrot and tomato fractions,
393 particle diameters expressed as $d_{(4,3)}$, $d_{(3,2)}$, and $d_{(v,0.5)}$ were considered (Table 2). In
394 accordance with previous experiments (Lopez-Sanchez et al., 2011), $d_{(4,3)}$ and $d_{(3,2)}$ were
395 calculated per peak. As for the cell cluster fraction of carrot and tomato most changes were
396 relevant to the large particle fraction, the latter solely will be further discussed. Similarly, in
397 the chromoplast-enriched fractions of carrot and tomato the smaller peak was not considered
398 due to its negligible contribution to the total volume. In general, both the volume-based and
399 area-based diameters, as well as $d_{(v,0.5)}$ showed similar trends with respect to the different
400 fractions (Table 2). Specifically, the particle diameters of carrot and tomato cell clusters
401 progressively decreased with increasing homogenisation intensity. On the other hand, the
402 particle diameters of the chromoplast-enriched fractions of carrot and tomato decreased up to
403 30 MPa, and no further changes could be observed at higher pressure levels.
404 HPH is well known to induce mechanical disruption of food matrices, the extent of disruption
405 being dependent on the homogeniser design (Perrier-Cornet, Marie, & Gervais, 2005). Thus,
406 the decrease in particle size depicted in Fig 2 and Table 2 was attributed to particle
407 deformation and breakage during HPH. (Floury, Legrand, &Desrumaux, 2004; Schultz et al.,
408 2004). The intercellular material (such as carotenoids), that is released following cell
409 disruption, is subjected to the same mechanical forces (Lee et al., 2009), resulting in its

enhanced contact with the bulk fluid. This eventually resulted in an increased mass transfer of the carotenoids to the oil phase (Bravo, 2011). Moreover, as it is known that shear stress leads to oil droplet deformation which increases their specific surface area (Floury, Desrumaux, & Lardières, 2000). this could have further facilitated their interaction with carotenoids. In this context, the increase in carotenoid concentration in the oil after HPH (Table 1), which translated into the increase in carotenoid transfer from the matrix to the oil (Fig 1), can be a reflection of not only the extent of carotenoid release following matrix disruption by HPH but also the influence of HPH as the driving force for carotenoid transfer into oil.

3.3 Relation between particle size and percentage carotenoid transfer

The relation between carotenoid transfer and changes in particle diameter expressed as $d_{(4,3)}$ due to HPH, in cell clusters and chromoplast-enriched fractions of both carrot and tomato, was investigated by linear regression analysis. Results are illustrated in Fig 3 and the corresponding parameter estimates are given in Table 3. Regression analysis (Fig 3) revealed a negative correlation between carotenoid transfer and particle size as influenced by HPH. Thus carotenoid transfer from the matrix increased with decreasing particle size. Confirming previous results (Fig 1) carotenoid transfer depended on the type of carotenoid on the one hand and the isolated fraction on the other hand. It can also be observed that the dependence of carotenoid transfer on particle size reduction as influenced by high pressure homogenisation was higher in the chromoplast-enriched compared to the cell cluster fractions, irrespective of matrix.

This is indicated by the differences in the slopes (Table 3) obtained for the linear regression models between cell clusters and chromoplast-enriched fractions within each matrix. In the cell cluster fractions from the tomato matrix, higher but steeper β-carotene slope values compared to lycopene (Table 3) were obtained indicating that the former carotenoid could be

435 transferred more efficiently. This observation highlights the influence of carotenoid
436 hydrophobicity on their transfer to oil upon HPH. Similarly, slope values obtained for α-
437 carotene and β-carotene in the carrot matrix are a reflection of the similarities in
438 hydrophobicity between the two carotenoids. As for the chromoplast-enriched fractions, in
439 spite of the higher slope values in the carrot matrix compared to the chromoplast-enriched
440 fractions from tomato (Table 2), the results in Fig 3 confirm the effect of the matrix on the
441 extent of carotenoid transfer to oil. This suggests that HPH can significantly increase
442 lycopene transfer to oil in the tomato matrix when intact cell walls are disrupted in the matrix
443 prior to adding the oil emulsion. Based on this observation, the increase in carotenoid transfer
444 depicted in Fig 1 from 30 to 100 MPa can be explained by the increased contact between the
445 released carotenoids and the oil droplets. In addition, the lower percentage transfer of
446 lycopene from tomato cell clusters (Fig 1C) compared to the values obtained for α- and β-
447 carotene from carrot cell clusters (Fig 1A) can be a consequence of the modification of the
448 tomato matrix that has been reported to possibly entrap lycopene thus hindering its release
449 (Colle et al., 2010b; Svelander et al.).
450 It is important to note that the applied pressure not only facilitates particle disruption but can
451 also change the properties of the continuous phase, namely the oil-in-water emulsion. As a
452 result, understanding the dynamic behaviour of the oil phase could be important for practical
453 applications when more concentrated oil-in-water dispersions are considered.
454 4. Conclusions
455 The present work evaluated the effect of high pressure homogenisation (HPH) on the transfer
456 of carotenoids from tomato and carrot-based matrices to the oil phase. Carotenoid transfer to
457 oil as a function of increasing homogenisation intensity presented an asymptotic behaviour. A
458 selective transfer of a particular carotenoid depending on the hydrophobicity of the
459 carotenoids could be observed in the cell cluster fractions solely, independently on the

matrix. The presence of cell walls in tomato cell cluster fractions was a limiting factor for carotenoid transfer to oil during high pressure homogenisation. The results indicate that under the conditions used in this study, HPH is efficient in transferring carotenoids to oil. In this way, the problem of reduced transfer to oil during digestion of some carotenoids is eliminated and this can have an influence on their bioaccessibility. Therefore, the results demonstrated a possible application of HPH as a valuable tool for improving the transfer of carotenoids to oil and pave the way for developing food products with enhanced levels of bioaccessible carotenoids.

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Captions

Table 1 - Concentrations (μg/g_{oil}) of lycopene, α-carotene and β-carotene from untreated samples (control) and high pressure homogenised cell clusters and chromoplast enriched fractions of carrot and tomato.

Table 2 - Particle size expressed as $d_{v,0.5}$, $d_{(4,3)}$ and $d_{(3,2)}$ of tomato and carrot fractions non-homogenised (control) and homogenised at 10, 30, 50, 70, 100 MPa.

Table 3 - Parameter estimates of the linear regression models describing the relation between carotenoid transfer and particle diameter (expressed as $d_{(4,3)}$) as influenced by high pressure homogenisation.

Fig. 1 - Carotenoid percentage transfer to the oil phase upon high pressure homogenisation. Carrot cell clusters (A) chromoplast-enriched fractions (B): (■) *α*-carotene (■) *β*-carotene. Tomato cell clusters (C) and chromoplast-enriched fractions (D): (▨) *β*-carotene; (□) lycopene. (C) Control = untreated sample. Different letters within each fraction for each carotenoid indicates significant differences (P<0.05).

Fig. 2 - Particle size distribution of: (A) carrot cell clusters, (B) carrot chromoplast-enriched fractions, (C) tomato cell clusters, (D) tomato chromoplast-enriched fractions homogenised at 10 MPa (——), 30 MPa (——), 50 MPa (——), 70 MPa (——), 100 MPa (——) and of the oil in water emulsion (——), untreated cell clusters (——) and untreated chromoplast-enriched fractions (-----).

Fig. 3 - Carotenoid transfer to oil as a function of particle size in (A) cell cluster fractions (B) chromoplast-enriched fractions of both carrot and tomato. (●) lycopene, (○) tomato *β*-carotene (□) carrot *α*-carotene (■) carrot *β*-carotene.

Table 1 – Concentrations (μg/g_{oil}) of lycopene, α-carotene and β-carotene from untreated samples (control) and high pressure homogenised cell clusters and chromoplast enriched fractions of carrot and tomato.

Matrix	Fraction	Carotenoid	control	10 MPa	30 MPa	50 MPa	70 MPa	100 MPa
Carrot	Cell clusters	*α*-carotene (μg/goil)	0.3±0.04^{a}	4.3 ± 0.4^{b}	13.6 ± 1.8^{c}	20.0 ±0.9^{d}	22.4±1.7^{d}	23.2 ±2.3^{d}
		β-carotene (μg/goil)	0.7 ±0.02^{a}	10.7±0.9^{b}	29.8±3.1^{c}	41.9±3.9^{d}	47.0±1.8^{e}	47.3±5.3^{e}
	Chromoplasts	*α*-carotene (μg/goil)	0.6±0.04^{a}	23.7±2.9^{b}	40.4±3.6^{c}	52.1±2.8^{d}	55.4 ±4.1^{d}	54.2±5.5^{d}
		β-carotene (μg/goil)	1.1±0.1^{a}	38.1±1.4^{b}	66.7±3.5^{c}	83.9±7.2^{d}	89.0±2.5^{d}	94.1±3.3^{d}
Tomato	Cell clusters	*β*-carotene (μg/goil)	0.2±0.04^{a}	2.5±0.4^{b}	5.4±1.5^{c}	7.9±0.4^{d}	8.9±1.9^{d}	9.1±1.1^{d}
		Lycopene (μg/goil)	1.1±0.04^{a}	9.2±0.3^{b}	17.7±3.1^{c}	22.0±1.3^{d}	25.7±2.3^{e}	23.6±1.7^{ed}
	Chromoplasts	*β*-carotene (μg/goil)	0.3±0.1^{a}	8.7±1.3^{b}	14.0±1.6^{c}	16.8±0.7^{d}	18.3±2.4^{ed}	18.1±1.3^{ed}
		Lycopene (μg/goil)	1.2±0.1^{a}	25.6±1.6^{b}	42.1±2.8^{c}	53.5±4.4^{d}	55.9±3.2^{d}	56.6±2.9^{d}

Values are means **± SD (N = 6)**
Significant differences within a row are indicated with different letters (P<0.05).

662 **Table 2 - Particle size expressed as $d_{v,0.5}$, $d_{(4,3)}$ and $d_{(3,2)}$ of tomato and carrot fractions non-homogenised**
663 **(control) and homogenised at 10, 30, 50, 70, 100 MPa.**

Matrix	Fraction	Particle size (μm)	control	10 MPa	30 MPa	50 MPa	70 MPa	100 MPa
Carrot	Cell clusters	$d_{(v,0.5)}$	254.2±12.6 ^{a}	236.4±11.8 ^{ab}	155.4±7.7 ^{c}	121.9±6.1 ^{d}	101.4±5.0 ^{e}	77.4±3.8 ^{f}
		$d_{(4,3)}$	265.3±13.2 ^{a}	250.5±12.5 ^{a}	167.5±8.3 ^{b}	128.3±6.4 ^{c}	107.7±5.4 ^{d}	83.2±4.1 ^{e}
		$d_{(3,2)}$	198.9±4.9 ^{a}	176.1±8.8 ^{b}	112.8±5.6 ^{c}	87.8±4.4 ^{d}	72.0±3.6 ^{e}	60.1±3.0 ^{f}
	Chromoplasts	$d_{(v,0.5)}$	3.90±0.02 ^{a}	1.30±0.03 ^{b}	1.18±0.03 ^{c}	1.07±0.03 ^{d}	1.01±0.02 ^{d}	1.05±0.02 ^{d}
		$d_{(4,3)}$	11.07±2.30 ^{a}	1.36±0.18 ^{b}	1.09±0.14 ^{c}	1.03±0.13 ^{c}	0.95±0.12 ^{cd}	0.84±0.11 ^{d}
		$d_{(3,2)}$	7.95±1.20 ^{a}	0.92±0.14 ^{b}	0.74±0.11 ^{bc}	0.69±0.11 ^{cd}	0.64±0.10 ^{cd}	0.56±0.09 ^{e}
Tomato	Cell clusters	$d_{(v,0.5)}$	448.4±14.2 ^{a}	432.9±13.7 ^{a}	247.5±7.8 ^{b}	197.4±6.3 ^{c}	168.5±5.3 ^{d}	104.3±3.2 ^{e}
		$d_{(4,3)}$	479.1±15.2 ^{a}	451.2±15.3 ^{a}	273.0±8.7 ^{b}	237.7±7.5 ^{cd}	241.4±7.7 ^{cd}	150.7±4.8 ^{e}
		$d_{(3,2)}$	3252.7±8.0 ^{a}	307.7±9.8 ^{b}	139.2±4.4 ^{c}	114.3±3.6 ^{d}	99.6±3.2 ^{e}	72.6±2.3 ^{f}
	Chromoplasts	$d_{(v,0.5)}$	4.58±0.15 ^{a}	1.31±0.02 ^{b}	1.22±0.02 ^{c}	1.19±0.12 ^{cd}	1.21±0.15 ^{c}	1.12±0.02 ^{e}
		$d_{(4,3)}$	13.78±0.51 ^{a}	1.07±0.12 ^{bc}	1.03±0.12 ^{bc}	0.90±0.11 ^{c}	0.91±0.11 ^{c}	0.91±0.11 ^{c}
		$d_{(3,2)}$	10.60±0.40 ^{a}	0.96±0.11 ^{b}	0.93±0.11 ^{cd}	0.81±0.09 ^{d}	0.81±0.09 ^{d}	0.81±0.09 ^{d}

Values are means ± SD (N = 4)

For the cell clusters fractions, values relevant to the large particles are reported.

For each matrix, different superscript letters in the same column indicate significant differences at $P < 0.05$.

664

665 **Table 3 - Parameter estimates of the linear regression models describing the relation between carotenoid**
666 **transfer and particle diameter (expressed as $d_{(4,3)}$) as influenced by high pressure homogenisation.**

Matrix	Fraction	Carotenoid	Slope	R^2
Carrot	Cell clusters	α-carotene	-0.378±0.023	0.99
		β-carotene	-0.312±0.026	0.98
	Chromoplasts	α-carotene	-103.68±20.24	0.9
		β-carotene	-93.65±11.94	0.95
Tomato	Cell clusters	β-carotene	-0.091±0.015	0.82
		Lycopene	-0.054±0.012	0.92
	Chromoplasts	β-carotene	-178.9±21.92	0.87
		Lycopene	-119.7±13.58	0.9

667

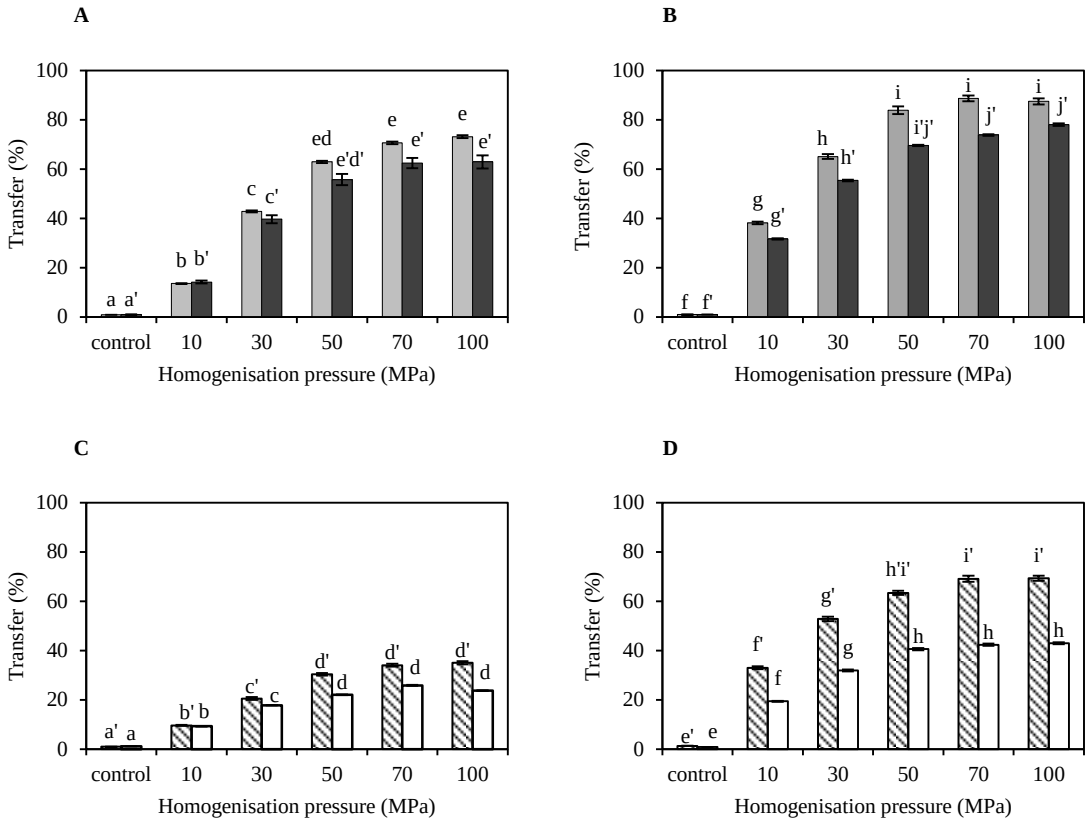
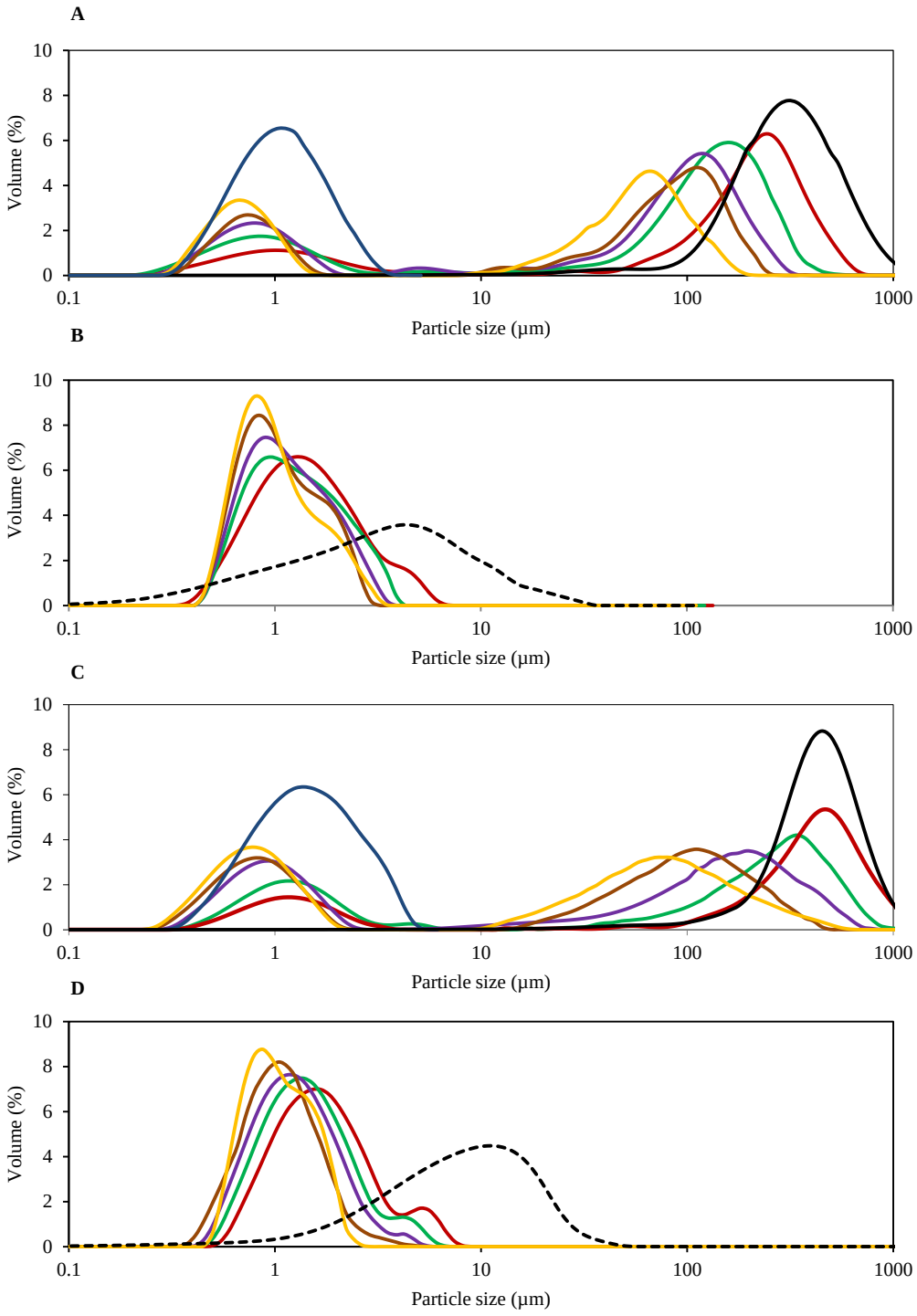
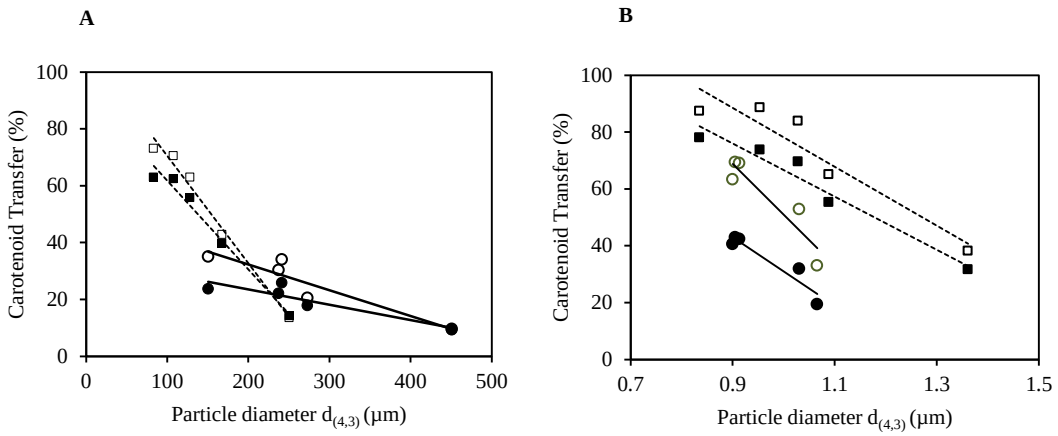

A
B
C
D
Transfer (%)
Homogenisation pressure (MPa)
control
10
30
50
70
100
a a'
b b'
c c'
ed e'd'
e e'
e e'
f f'
g g'
h h'
i i'j'
i j'
i j'
a' a
b' b
c' c
d' d
d' d
d' d
e' e
f' f
g' g
h'i' h
i' h
i' h

Fig. 1


A
Volume (%)
Particle size (µm)
B
Volume (%)
Particle size (µm)
C
Volume (%)
Particle size (µm)
D
Volume (%)
Particle size (µm)

670

671 **Fig. 2**



672

673 **Fig. 3**

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